Topology of Epoxide Hydrolase in the Membrane of the Endoplasmic Reticulum

JANERIC SEIDEGÅRD,[a] JOSEPH W. DePIERRE,[a] THOMAS M. GUENTHNER[b] and FRANZ OESCH[c]

[a] Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden, [b] Department of Pharmacology, University of Illinois Medical Center, Chicago, Ill., U.S.A. and [c] Institute of Pharmacology, University of Mainz, Mainz, Federal Republic of Germany

As has been most clearly demonstrated in the case of mitochondrial inner membranes, chloroplast lamellae, and certain enzymes on the endoplasmic reticulum,[1] the topology of membrane-bound enzymes often has important functional consequences. At present relatively little is known about the topology of epoxide hydrolase in the membrane of the endoplasmic reticulum. A detailed subfractionation study performed earlier in our laboratory[2] suggested that epoxide hydrolase does not form a stoichiometric complex with cytochrome P-448, at least not after induction with 3-methylcholanthrene. The topology of microsomal epoxide hydrolase has also been investigated using proteases[2] and the results have led to the tentative conclusion that little or none of this enzyme is exposed at the outer microsomal surface (=the cytoplasmic surface of the endoplasmic reticulum).

Each of the individual techniques used to investigate the transverse topology of membrane-bound enzymes has its limitations.[1] For instance, epoxide hydrolase may be exposed at the outer microsomal surface and still be resistant to attack by protease. Therefore, we felt that it was important to study the transverse topology of this enzyme using complementary approaches.

In the present experiments microsomal epoxide hydrolase was labelled using lactoperoxidase, H_2O_2, and $^{125}I^-$. This system results in the iodination of proteins, chiefly on tyrosine and histidine residues, exposed at the surface of intact membrane vesicles, since lactoperoxidase is far too large to penetrate across biological membranes.[3] In order to also label proteins exposed at the inner microsomal surface we made the microsomal vesicles permeable to lactoperoxidase with 0.05% deoxycholate.[4] As a control for achieving labelling of all groups in epoxide hydrolase which can be iodinated by lactoperoxidase the isolated lipid and detergent-depleted enzyme was iodinated under similar conditions. Finally, in order to investigate the role of membrane phospholipids in determining the transferase topology of epoxide hydrolase, the enzyme was purified to homogeneity and reincorporated into liposomes composed of phosphatidylcholine or total rat liver microsomal phospholipids. The topology of the enzyme in these liposomes was subsequently compared to its topology in microsomes using the same iodination procedures.

Liver microsomes were prepared from male Sprague-Dawley rats weighing 200–220 g (obtained from Versuchstierzuchtanstalt, WIGA, Sulzfield, Federal Republic of Germany). The liver was homogenized in 3 volumes 1.15% KCl containing 10 mM sodium phosphate, pH 7.4. The homogenates were centrifuged at 10 000 *g* for 10 min and the resulting supernatant fraction was centrifuged at 100 000 *g* for 1 h. The microsomal pellets were subsequently resuspended in 1.15% KCl–10 mM sodium phosphate, pH 7.4, to give a final protein concentration of 5 mg/ml.

Microsomal epoxide hydrolase was purified to apparent homogeneity from rats treated with *trans*-stilbene oxide essentially according to the procedure of Bentley and Oesch,[5] but with certain modifications designed to achieve higher yields and obtain an enzyme perparation containing smaller amounts of detergent.[6]

Epoxide hydrolase activity towards styrene oxide was determined using the procedure reported earlier.[7]

Total microsomal phospholpids were extracted from rat liver microsomes according to Bligh and Dyer[8] and were stored, as was egg yolk phosphatidylcholine (Sigma Chemical Co., St. Louis, Missouri, USA), in chloroform solution. After evaporation of the chloroform, phospholipids were mixed with purified epoxide hydrolase in a ratio of 10:1, w:w, in the presence of 1% sodium cholate. This mixture was then submitted to two chromatographic steps:[9] both columns were coated with phospholipids and with bovine serum albumin before use. Upon chromatography on Sephadex G-50 virtually all protein and phospholipid eluted in the void volume, while nearly 99% of the cholate present was retarded on the column. Subsequent chromatography on Sepharose 4B revealed that nearly all the protein was associated with the smaller phospholipid vesicles. In a control experiment [^{14}C]-phosphatidylcholine was added to the original mixture and monitored through the two chromatographic steps to further confirm that epoxide hydrolase had indeed been incorporated into phospholipid vesicles by this procedure.

Microsomes, reconstituted lipid vesicles or isolated microsomal epoxide hydrolase were

0302-4369/82/080555-03$02.50
© 1982 Acta Chemica Scandinavica

Table 1. Iodination of epoxide hydrolase in intact microsomes and after incorporation into liposomes.[a]

System	Intact (%)	Disrupted with deoxycholate (%)
Epoxide hydrolase in intact microsomes	20–25	20–25
Purified epoxide hydrolase incorporated into liposomes of phosphatidylcholine	40–45	80–90
Purified epoxide hydrolase incorporated into liposomes of total rat liver microsomal lipids	25–30	25–30

[a] The figures in the table are the percentages of the total iodinatable groups in epoxide hydrolase (as determined by iodination of the purified, lipid- and detergent-depleted enzyme) labeled in the different systems using lactoperoxidase, H_2O_2, and $^{125}I^-$. For further details see the text.

iodinated[10] in a reaction mixture containing 50 mM potassium phosphate, pH 7.2, 0.2 mg protein/ml, 0.3 μM lactoperoxidase and carrier-free ^{125}I (0.37 – 1.85 MBq/ml). The reaction was started by the addition of H_2O_2 to give a final concentration of 4 μM. This addition was repeated 3 more times during the incubation, which was carried out for 30 min at 20 °C in the dark and terminated by the addition of $Na_2S_2O_3$ and KI to give final concentrations of 0.1 mM and 10 μM, respectively. (The concentrations of lactoperoxidase and H_2O_2 were determined by measuring the optical density at 412 nm and 230 nm, respectively. The absorption coefficients used were 114 mM^{-1} cm^{-1}[11] and 72.4 M^{-1} cm^{-1},[12] respectively).

Iodinated microsomes and phospholipid vesicles, were collected by centrifugation for 30 min at 105,000 g. The pellet was suspended in 50 mM Tris-Cl, pH 7.6, and this procedure repeated three times to remove unbound ^{125}I. The microsomes were then solubilized with 1 % sodium cholate and incubated at room temperature for 3 h with rabbit antibodies against rat liver microsomal epoxide hydrolase (1 mg antibody protein/mg microsomal protein). Goat antibodies against immunoglobulins (1 – 2 mg goat antibodies/40 μg rabbit IgG) were subsequently added to the mixture and the incubation continued overnight at 4 °C.

The epoxide hydrolase – rabbit anti – epoxide hydrolase – goat anti – IgG complex was precipitated by centrifugation at 20 000 *g* for 10 min. This precipitate or reconstituted vesicles precipitated with TCA were then dissolved by boiling for 1 – 2 min in 6 % sodium dodecyl sulfate and epoxide hydrolase was separated from the immunoglobulins by SDS-slab gel electrophoresis according to Laemmli.[13] Purified microsomal epoxide hydrolase was separated directly from the reaction mixture used for iodination by SDS-slab gel electrophoresis. The gels were stained with Coomassie blue and the band corresponding to epoxide hydrolase was cut out and the amount of ^{125}I it contained determined by scintillation counting.

It can be seen from Table 1 that when intact microsomes are incubated with lactoperoxidase, H_2O_2, and $^{125}I^-$, 20 – 25 % of the groups in epoxide hydrolase which can be iodinated are labelled. Assuming a random distribution of such groups along the peptide chain of this enzyme, this finding suggests that 1/5 – 1/4 of the epoxide hydrolase molecule is exposed at the outer microsomal surface (= the cytoplasmic surface of the endoplasmic reticulum). There is no increase in the iodination of epoxide hydrolase by lactoperoxidase, H_2O_2, and $^{125}I^-$ when the microsomal vesicles are made leaky with deoxycholate (Table 1), suggesting that little or none of this protein is exposed at the inner microsomal surface (= the luminal surface of the endoplasmic reticulum).

This indicated topology of the microsomal epoxide hydrolase – *i.e.*, the exposure of a small portion of the protein at the cytoplasmic surface and the localization of most of the polypeptide chain in the membrane itself – agrees well with earlier results[14] (see above). Such a topology might provide the microsomal epoxide hydrolase with easy access to epoxides dissolved in the phospholpid bilayer of the endoplasmic reticulum and to epoxides dissolved in the cytoplasm or bound to soluble proteins. It should be remembered that since we used antibodies directed specifically towards a single, homogeneous form of microsomal epoxide hydrolase to determine the extent of iodination (see above), our results are relevant only to this form of the enzyme. It has been reported that there are at least three different microsomal epoxide hydrolases[15] and it may be that the other forms have different transverse topologies in the membrane of the endoplasmic reticulum. However, it may be that the antibody used here can also cross-react with other forms of microsomal epoxide hydrolase.

Table 1 also documents the iodination of purified epoxide hydrolase which has been reincorporated into liposomes composed of phosphatidylcholine or of total rat liver microsomal lipids. After incorporation into liposomes composed of egg yolk phosphatidylcholine 40–45% of the groups that can be iodinated in epoxide hydrolase are labelled by lactoperoxidase, H_2O_2, and $^{125}I^-$. This is considerably more than in the case of intact microsomes. Furthermore, after disruption of these liposomes with deoxycholate almost all of the groups can be labelled (Table 1). Thus, in liposomes formed from egg yolk phosphatidylcholine epoxide hydrolase is almost totally exposed at the membrane surfaces and is also randomly distributed between the inner and outer surfaces. This pattern is very different from that seen with intact microsomes.

On the other hand, after incorporation into liposomes composed of total rat liver microsomal lipids, the topology of epoxide hydrolase as revealed by the approach employed here is essentially the same as that seen in intact microsomes (Table 1). These findings suggest that membrane phospholipids have an important role to play in the transverse topology of epoxide hydrolase in the endoplasmic reticulum.

Acknowledgements. J. Seidegård was supported by travel grants from the Swedish Cancer Society and the Swedish Natural Science Research Council. These investigations were funded by grants from the Swedish Cancer Society and the National Cancer Institute (Grant 1 RO ICA 26251-03), Bethesda, Md., USA.

1. DePierre, J. W. and L. Ernster, L. *Ann. Rev. Biochem. 46* (1977) 201.
2. Seidegård, J., Moron, M. S., Eriksson, L. C. and DePierre, J. W. *Biochim. Biophys. Acta 543* (1978) 29.
3. Morrison, M. *Methods Enzymol. 32* (1974) 103.
4. Kreibich, G., Pascale, D. and Sabatini, D. D. *J. Cell. Biol. 58* (1973) 436.
5. Bentley, P. and Oesch, F. *FEBS Lett. 59* (1975) 291.
6. Guenthner, T. M., Bentley, P. and Oesch, F. *Methods Enzymol.* 77 (1981) 344.
7. Oesch, F., Jerina, D. M. and Daly, J. *Biochim. Biophys. Acta 227* (1971) 685.
8. Bligh, E. G. and Dyer, W. J. *Can. J. Biochem. Physiol. 37* (1959) 911.
9. Halpert, J., Glaumann, H. and Ingelmann-Sundberg, M. *J. Biol. Chem. 254* (1979) 7434.
10. Gellerfors, P. and Nelson, B. D. *Eur. J. Biochem. 80* (1977) 275.
11. Morrison, M. and Bayse, G. S. *Biochemistry 9* (1970) 2995.
12. George, P. *Biochem. J. 54* (1953) 267.
13. Laemmli, U. K. *Nature 227* (1970) 680.
14. Oesch, F. and Bentley, P. *Nature 259* (1976) 53.
15. Guengerich, F. P., Wang, P., Mitchell, M. B. and Mason, P. S. *J. Biol. Chem. 254* (1979) 12248.

Received March 5, 1982.